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# Compartmentalization of the Somite and Myogenesis in Chick Embryos Are Influenced by *Wnt* Expression

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**Muscles of the body and bones of the axial skeleton derive from specialized regions of somites. Somite development is influenced by adjacent structures. In particular, the dorsal neural tube and the overlying ectoderm have been shown to be necessary for the induction of myogenic precursor cells in the dermomyotome. Members of the *Wnt* family of signaling molecules, which are expressed in the dorsal neural tube and the ectoderm, are postulated to be responsible for this process. It is shown here that ectopically implanted *Wnt-1*-, *-3a*-, and *-4*-expressing cells alter the process of somite compartmentalization *in vivo*. An enlarged dorsal compartment results from the implantation of *Wnt*-expressing cells ventrally between the neural tube/notochord and epithelial somites, at the expense of the ventral compartment, the sclerotome. Thus, ectopic *Wnt* expression is able to override the influence of ventralizing signals arising from notochord and floor plate. This shift of the border between the two compartments was identified by an increase in the domain of *Pax-3* expression and a complete loss of *Pax-1* expression in somites close to the ectopic *Wnt* signal. The expanded expression of *MyoD* and desmin provides evidence that it is the myotome which increases as a result of Wnt signaling. *Paraxis* expression is also drastically amplified after implantation of *Wnt*-expressing cells indicating that Wnts are involved in the formation and maintenance of somite epithelium and suggesting that *Paraxis* is activated through Wnt signaling pathways. Taken together these results suggest that ectopic Wnts disturb the normal balance of signaling molecules within the somite, resulting in an enhanced recruitment of somitic cells into the myogenic lineage.** © 2000 Academic Press

***Key Words:*** **chick embryo; *Wnts;* epithelialization; somite compartmentalization; myogenesis; *Paraxis; Pax-3; Pax-1; MyoD*.**

## INTRODUCTION

Skeletal muscle of the body derives from the somites that flank the neural tube and bud off in pairs from the unsegmented paraxial mesoderm in rostral to caudal progression. The newly formed somites are epithelial spheres and subsequently become patterned to form the sclerotome ventrally and the dermomyotome dorsally. The sclerotome undergoes an epitheliomesenchymal transition and gives rise to the future vertebrae and ribs. The dermomyotome remains epithelial and becomes subdivided into dermomyotome and myotome. Myogenic precursor cells detach from, and invaginate under, the epithelial dermomyotome to form the myotome (Denetclaw *et al.*, 1997). Within the dermomyotome two different myogenic lineages arise (Ordahl and Le Douarin, 1992; Kablar *et al.*, 1999). The medial part of the dermomyotome gives rise to epaxial muscle (trunk muscle) while the lateral part gives rise to the hypaxial muscle which will constitute the muscle of the limbs and the ventrolateral body wall (for review see Christ and Ordahl, 1995; Brand-Saberi *et al.*, 1996).

The promoting effect of dorsal neural tube and ectoderm on myogenesis coincides with the expression pattern of the *Wnt* family of genes (newly named by Nusse *et al.*, 1991). In the neural tube and in the ectoderm diverse *Wnts* are expressed (Shackleford and Varmus, 1987; Wilkinson *et al.*, 1987; Roelinck and Nusse, 1991; Parr *et al.*, 1993; Hollyday *et al.*, 1995; see also Fig. 1B). *Wnt-1* and *-3a* are expressed in the dorsal neural tube, and *Wnt-4* and *-6* in the ectoderm. *Wnts* play important roles in the determination of cell fate, proliferation, and cell survival (Nusse, 1988; McMahon,

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1992; McMahon and McMahon, 1989; Nusse and Varmus, 1992; Dickinson *et al.*, 1995; Yamaguchi *et al.*, 1999).

The *Wnt* genes encode secreted glycoproteins that bind to the family of Frizzled-receptors (Fz), which in turn transduce the signal into the target cells (Chan *et al.*, 1992; Bhanot *et al.*, 1996; Yang-Snyder *et al.*, 1996; He *et al.*, 1997). This leads to an accumulation of unphosphorylated β-catenin, the key mediator in a very complex signaling network (Gumbiner, 1996; Willert and Nusse, 1998; Martinez Arias *et al.*, 1999; Polakis, 1999). β-Catenin subsequently enters the nucleus where it binds to TCF/LEF transcription factors which ultimately induce the downstream Wnt target genes (Huber *et al.*, 1996a; Behrens *et al.*, 1996; Molenaar *et al.*, 1996; Clevers and van de Wetering, 1997; McKendry *et al.*, 1997; Bienz, 1998). These properties make the dorsally expressed *Wnts* excellent candidates for effectors of dorsal somite development, including the initiation of somite epithelialization, dermomyotome formation, and myogenesis.

The epithelial structure of the newly formed somite, as well as that of the dermomyotome, may be influenced by Wnts, which have been suggested to induce an epithelial morphology via β-catenin, in its role as a cell adhesion molecule (Hinck *et al.*, 1994; Gumbiner, 1996). Somite epithelialization, and later differentiation of the dermomyotome, can be followed by the expression of *Paraxis* (Burgess *et al.*, 1996).

*Wnts* were shown to be required for induction of myogenesis in the paraxial mesoderm in coculture experiments (Stern *et al.*, 1995; Münsterberg *et al.*, 1995; Fan *et al.*, 1997; Reshef *et al.*, 1998). The induction of *myf-5* expression was attributed to the neural-tube-derived Wnts, while *MyoD* expression was induced by ectodermally derived Wnts (Tajbakhsh *et al.*, 1998). Capdevila *et al.* (1998) enhanced Wnt signaling by retroviral mediated overexpression in the paraxial mesoderm and observed an enlargement of the *Pax-3*-expressing, dorsal somite compartment.

In this study we investigated the effect of ectopically expressed, dorsalizing signal molecules *(Wnt1/3a/4)* on somites *in vivo*. We focused on the questions of whether the Wnts are able to promote epithelialization, alter somite compartmentalization, and influence myogenesis. *Wnt*-expressing cells, when ectopically implanted between neural tube/notochord and ventral somite, produced an expansion of the dorsal, epithelially structured somitic compartment in which *Paraxis*, *Pax-3*, and *MyoD* were found to be expressed. That this expansion occurred at the expense of the ventral somitic compartment was shown by a loss of the sclerotomal marker, *Pax-1*, in affected somites.

## MATERIAL AND METHODS

### *Embryos*

Fertile eggs (White Leghorn) were obtained from a local breeder and incubated at 37.8°C and 80% relative humidity for 2 days up to HH-stages 11–14 (Hamburger and Hamilton, 1951).

**TABLE 1**

Implantation of Different Wnt-Secreting Cells Alters Normal Gene Expression

| | *Pax-3* | *Pax-1* | *Paraxis* | *MyoD* | *Desmin* |
|---|---|---|---|---|---|
| *Wnt-1* | 7/9 | 8/8 | 12/16 | 18/23 | 7/9 |
| *Wnt-3a* | 8/11 | 7/7 | 8/10 | 9/10 | 5/5 |
| *Wnt-4* | 6/6 | 4/4 | 4/4 | 4/4 | 4/5 |

*Note.* No. of embryos with altered gene expression/No. of operated embryos.

### *Cell Lines and Culture Conditions*

*Wnt*-expressing rat B1a fibroblasts and NIH3T3 cells were kindly provided by A. B. Lassar and A. Kispert. These cell lines were infected with a retroviral vector, as described previously (J. Kitajewski and Z. Zheng). LNCX-/Lac-Z-transfected fibroblasts were generated in the same manner and used as control cells. The cells were selected in G418 and grown under conditions as described by Münsterberg *et al.* (1995).

### *Operations*

Cell pellets were implanted into a prepared tunnel between the axial organs and epithelial somites by using a micropipette (Fig. 1A). The operated embryos were reincubated for approximately 20 h. The number of operated embryos is shown in Table 1.

### *Histology*

Embryos were fixed overnight in Serra's solution, dehydrated, and embedded in paraffin. Sections were cut at 8 μm and True red staining was carried out according to Romeis (1968).

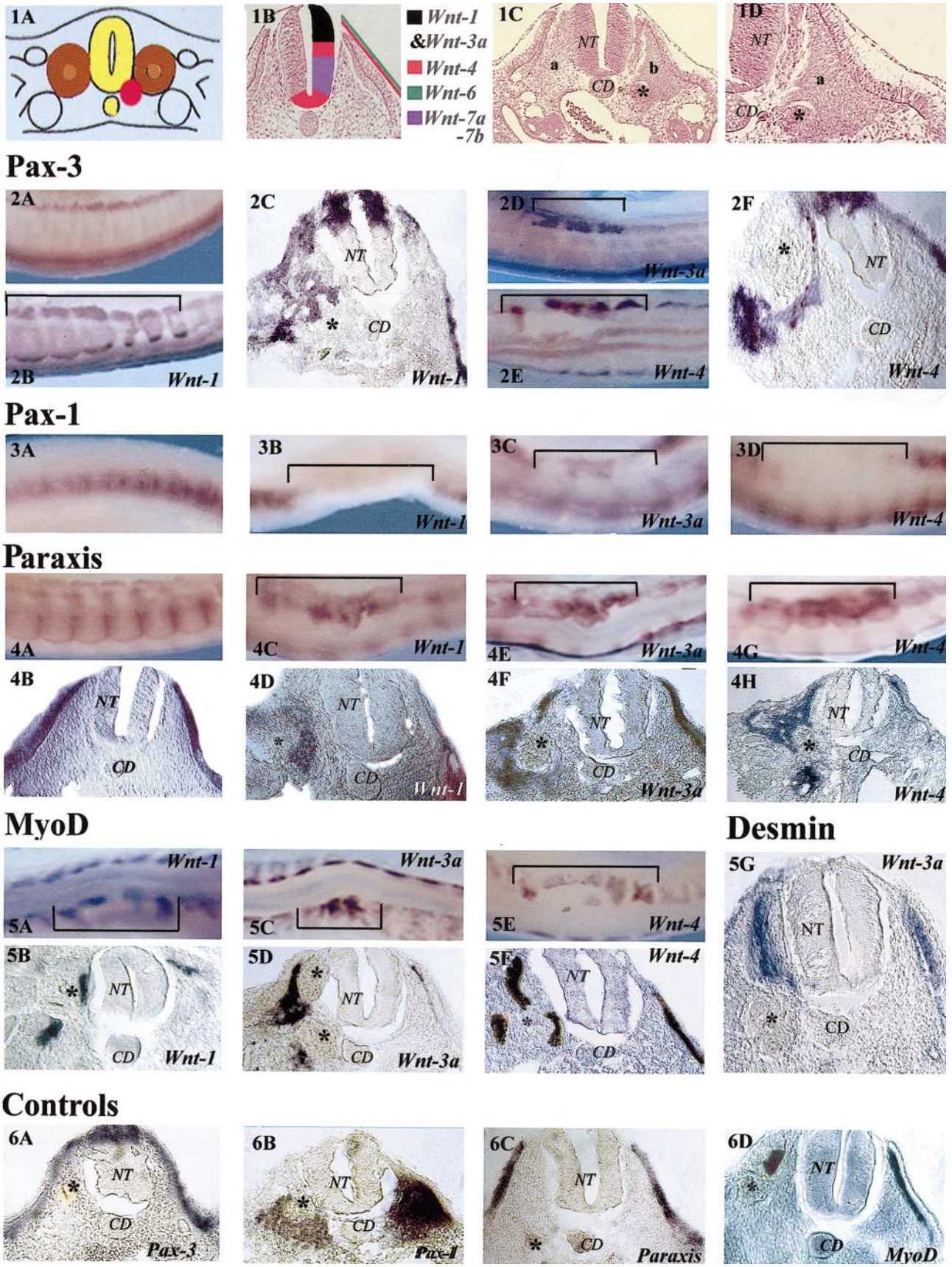
### *In Situ Hybridization*

Embryos were washed in PBT and fixed overnight in 4% PFA/PBT at 4°C. The antisense RNA probe was labeled with digoxigenin and whole-mount *in situ* hybridization was performed as described by Nieto *et al.* (1996). The following probes were used in this study: a probe of *Pax-1* (a 1.5-kb insert cloned into pBluesript II KS (C. Ebensperger)); a probe of *Pax-3* (a 1543-bp insert cloned into pGEM 72f (gift from M. Bronner-Fraser)); a probe of *Paraxis* (a 1380-bp insert cloned into pBSSK+ (gift from E. Olson)); and a probe of *MyoD* (a 1518-bp insert cloned into pKS+ Stratagene (Lin *et al.*, 1989)). The embryos were cryosectioned for further examinations.

## RESULTS

### *The Influence of Wnt-Expressing Cells on Somite Compartmentalization*

*Wnt-1*-expressing cells implanted between axial organs and epithelial somites of chick embryos at stage 12, as


1A
1B
Wnt-1
&Wnt-3a
Wnt-4
Wnt-6
Wnt-7a
-7b
1C
NT
a
b
CD
1D
Pax-3
2A
2B
Wnt-1
2C
2D
Wnt-3a
2E
Wnt-4
2F
Pax-1
3A
3B
3C
3D
Paraxis
4A
4B
4C
4D
4E
4F
4G
4H
MyoD
5A
5B
5C
5D
5E
5F
Desmin
5G
Controls
6A
Pax-3
6B
Pax-1
6C
Paraxis
6D
MyoD

shown in Fig. 1A, lead after a reincubation period of approximately 20 h to an enlarged myotome that was observed at the operation site but not at the same position in the contralateral control somites (Fig. 1C). The implanted *Wnt-1*-expressing cells are enclosed by myotomal tissue (Fig. 1D) and only single sclerotomal cells could be detected. The dermatome was not altered structurally.

### *Pax-3 Expression Is Up-Regulated by Wnt-Expressing Cells*

The implantation of *Wnt-1-*, *Wnt-3a-*, or *Wnt-4*-expressing fibroblasts leads to increased *Pax-3* expression in adjacent somites. The grafted cells are medially and laterally flanked by a domain of strong *Pax-3* expression at the

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**FIG. 1.** (A) Operation scheme: *Wnt*-expressing cells are grafted between axial organs and ventral epithelial somite; implanted Wnt cells, red; neutral tube/notochord, yellow; somites, brown. (B) Control embryo (HH-stage 18) with expression patterns of the diverse *Wnts.* Data collected from Parr *et al.* (1993), Hollyday *et al.* (1995), Marcelle *et al.* (1997), and Borycki *et al.* (1998). (C)Transverse section of a HH-stage 18 embryo shows an enlarged myotome-like compartment which surrounds implanted *Wnt-1*-expressing cells. True red staining: (a) mesenchyme; (b) epithelium (D) Same embryo as shown in (C); operation side; higher magnification. (a) epithelium. In C and D, implanted cells are marked by asterisks (cross-sections). NT, neural tube; CD, notochord.

**FIG. 2.** *Pax-3 in situ* hybridization. Whole-mount normal embryo; HH-stage 19. HH-stage 19 chick embryo after implantation of *Wnt-1*-expressing cells; somites show structure abnormalities. *Pax-3* is expressed throughout the whole somite and at higher levels medially and laterally. Corresponding sections of the embryo shown in (B) present an irregularly expanded expression domain; the cells are surrounded by *Pax-3*-positive tissue which is morphologically epithelially structured. Only the space between implant and notochord is left free. HH-stage 19 embryo after implantation of *Wnt-3a*-expressing cells; *Pax-3* expression is upregulated laterally in the somite at the transition to the upper limb. The whole mount of a HH-stage 18 chick embryo after implantation of *Wnt-4*-expressing cells displays a massive increase in *Pax-3* expression lateral of the cells. Corresponding sections of the embryo shown in (E) present the implanted *Wnt-4*-expressing cells lying quite dorsal in the somite. They are surrounded by *Pax-3*-positive tissue from each side. Especially strong expression is notable in the lateral part of the somite and even ventrally between cells and notochord an expression could be found. In B, D, and E, the operation site is marked by brackets (whole mounts); in C and F, implanted cells are marked by asterisks (cross-sections). NT, neural tube; CD, notochord.

**FIG. 3.** *Pax-1 in situ* hybridization. (A)Whole-mount staining of a normal chick embryo HH-stage 19. (B) Chick embryo after implantation of *Wnt-1*-expressing cells shows a complete absence of *Pax-1* at the operation site. (C) Chick embryo shows an altered *Pax-1* expression after implantation of *Wnt-3a*-expressing cells. *Pax-1* is down-regulated at the operation site. (D) The whole mount of a HH-stage 19 embryo presents a complete down-regulation of *Pax-1* at the site of implantation of *Wnt-4*-expressing cells. In B–D, the operation site is marked by brackets (whole mounts).

**FIG. 4.** *Paraxis in situ* hybridization. (A) Whole-mount specimen of a normal chick embryo; HH-stage 18. (B) Corresponding transverse section. (C) Whole mount of a HH-stage 19 embryo after implantation of *Wnt-1*-expressing cells. The embryo displays an irregular somitic structure and an augmented *Paraxis* expression at the operation site. (D) Corresponding transverse section; the graft is medially surrounded by *Paraxis*-positive tissue. (E) The HH-stage 19 embryo shows an up-regulation of *Paraxis* after implantation of *Wnt-3a*-expressing cells. (F) Corresponding sections of the embryo shown in (E) display the implant lying surrounded by a tissue with a high level of *Paraxis* expression. Even the ventral domain of the somite is labeled except for the space directly neighboring the notochord. After implantation of *Wnt-4*-expressing cells the embryo displays an extreme upregulated expression of *Paraxis.* The somites are *Paraxis* positive on the whole at the operation site. Sections of the same embryo as shown in (G) present an enlarged domain of *Paraxis* expression which neighbors the implant and is epithelially structured. Ventrally a supernumerary expression domain in proximity to the notochord can be found. In C, E, and G, operation site is marked by brackets (whole mounts); in B, D, F, and H, implanted cells are marked by asterisks (cross-sections). NT, neural tube; CD, notochord.

**FIG. 5.** *MyoD in situ* hybridization After implantation of *Wnt-1*-expressing cells an altered somitic structure could be found. Sections of the same embryo as shown in (A) present the cells located between two domains of *MyoD* expression. One of them lies next to the neural tube; the other one is located ventrally in quite close proximity to the notochord. The whole mount of this embryo displays a strong up-regulation of *MyoD* at the site of implantation of *Wnt-3a*-expressing cells; HH-stage 19. (D) Sections of the same embryo as shown in (C) present an enlarged *MyoD*-positive myotome-like compartment neighboring the implanted cells. Another *MyoD*-labeled domain could be found adjacent to the cells. Embryo (HH-stage 18) after implantation of *Wnt-4*-expressing cells; graft divides the somites, which are *MyoD* positive on the whole at the operation site. The corresponding sections show three supernumerary domains of *MyoD* expression. Desmin immunohistochemistry: HH-stage 19 chick embryo after implantation of *Wnt-3a*-expressing cells; the graft is surrounded by desmin-positive cells. The muscle-forming desmin-positive compartment is enlarged and occupies even the space next to the ventral neural tube and the notochord. In A, C, and E, the operation site is marked by brackets (whole mounts); in B, D, F, and G, implanted cells are marked by asterisks (cross-sections). NT, neural tube; CD, notochord.

**FIG. 6.** Control cells. (A) Transverse sections show no effect on *Pax-3* expression after control cell implantation. The cells could be seen between dermomyotome and notochord surrounded by unaltered sclerotomal mesenchyme. (B) HH-stage 18 embryo after implantation of control cells; no alteration of *Pax-1* expression could be observed on the operation side. (C) Transverse section presents a normal pattern of *Paraxis* expression after implantation of control cells. The cells are located in unaltered sclerotome. (D) The transverse section shows a shortened myotome on the operation side but no extra domains of *MyoD* expression. In all panels implanted cells are marked by asterisks (cross-sections). NT, neural tube; CD, notochord.

operation site (Figs. 2B–2F). In some instances, affected somites do not show the typical gap between the medial and the lateral dermomyotomal domains of *Pax-3* expression, and thus the entire dermomyotome appears to be *Pax-3* positive. In whole-mount preparations, an increase of *Pax-3* expression could be detected especially in the lateral part of the somite at the transition to the upper limb (Fig. 2D). In transverse sections, the extended *Pax-3* domain reaches more ventrally than in unaffected somites (Fig. 2F). Only the cells in the space between the grafted cells and the notochord did not express *Pax-3* (Fig. 2C).

### *Pax-1 Is Down-Regulated by Wnt-Expressing Cells*

After implantation of *Wnt-1-, Wnt-3a,* or *Wnt-4*-expressing cells there was a dramatic down-regulation of *Pax-1* in the ventral part of the somites. In all cases *Pax-1* expression is clearly reduced adjacent to the implanted cells and in some embryos there was a complete absence of expression (Figs. 3B–3D).

### *Paraxis Expression Is Up-Regulated by Wnt-Expressing Cells*

The implantation of *Wnt-1-, Wnt-3a-,* or *Wnt-4*-expressing cells leads to an increase in the domain of *Paraxis* expression (Figs. 4C–4H). Transverse sections showed that the grafted cells were surrounded by *Paraxis*-positive cells (Figs. 4D, 4F, and 4H). In some cases a ventral *Paraxis*-expressing domain was found close to the notochord (Fig. 4H).

### *MyoD Is Up-Regulated by Wnt-Expressing Cells*

Following implantation of *Wnt-1-, Wnt-3a-,* or *Wnt-4*-expressing cells, the region of *MyoD* expression was dramatically increased (Figs. 5C–5H). In some instances, the ectopic cells divided the myotome in two parts, both of which were clearly *MyoD* positive (Fig. 5D). In other cases the implants were surrounded by an extended *MyoD*-positive myotome (Figs. 5F and 5H). *In toto* the expression domain of *MyoD* on the operated side was more than twice the width as that found on the control side.

### *Control Cells Do Not Alter the Expression of Pax-1, Pax-3, Paraxis, MyoD, or Desmin*

To demonstrate that the up-regulation of *Pax-3, Paraxis, MyoD,* and desmin and the down-regulation of *Pax-1* was not due to a barrier effect of the implanted cells, which might inhibit normal distribution of signaling molecules arising from the neural tube/notochord, *LNCX*/Lac-Z control cells were implanted at the level of the epithelial somites between neural tube/notochord and somite. Despite a certain shortening of the dermomyotome, no alterations of the normal somite compartmentalization were found. The control cells showed no effect on the normal expression pattern of *Pax-3* (Fig. 6A), *Pax-1* (Fig. 6B), *Paraxis* (Fig. 6C), or *MyoD* (Fig. 6D).

## DISCUSSION

The present study demonstrates that ectopically implanted *Wnt-1-, -3a-,* or *-4*-expressing cells alter normal somite compartmentalization. The dorsal compartment became enlarged at the expense of the ventral compartment, as shown by the expansion of the dorsal marker, *Pax-3,* and the loss of *Pax-1* expression. The enhanced expression of *Paraxis* in the paraxial mesoderm points to an enlargement of somitic epithelium and of the dermomyotome. This enlarged compartment produced increased numbers of myogenic cells leading to an expansion of the myotome.

### *Somite Compartmentalization and Myogenesis Are Influenced by Wnts*

Previous studies indicate that one role of signals emerging from the dorsal neural tube is to establish dorsal/ventral polarity in the somite. While Christ *et al.* (1992) described some compartmentalization of somites after ablation of the neural tube, these somites were unable to give rise to muscle. This observation emphasizes the function of the neural tube in dorsoventral patterning of the somites and in inducing myogenesis. The surface ectoderm was able to partially rescue the loss of neural tube signals, but was unable to initiate and maintain myogenesis (Bober *et al.,* 1994a; Borman and Yorde, 1994). The neural tube and surface ectoderm are both sources of Wnts, but each expresses different members of the family. The difference in Wnts expressed by the surface ectoderm and the neural tube could account for the different effect on myogenesis evoked by these two tissues.

In our experiments, no difference on somite development was observed between neural tube- (Wnt-1 and -3a) and surface ectoderm- (Wnt-4) derived Wnts. One explanation for the strong myogenic influence of the surface ectoderm-derived Wnt-4 signal may lie in the level of expression produced by positioning the Wnt-secreting cells close to the ventral part of the somite. Stern *et al.* (1995) demonstrated that the dosage of Wnt-1 plays a critical role in initiating myogenesis. It is likely that the thin ectodermal layer is not able to provide the same dose of Wnt-4 as the graft used in our experiments and that a presumably high level of Wnt-4 can augment neural-tube-derived Wnts to increase myogenesis to the level we observed. A second possibility is that the *Wnts* which we ectopically expressed in the paraxial mesoderm are able to induce other *Wnts* in the adjacent neural tube, which in turn signal the adjacent somites. That Wnts can induce one another was shown by Marcelle *et al.* (1997), in which Wnt-1 and -3a induced the expression of *Wnt-11* in the medial part of the dermomyotome.

Capdevila *et al.* (1998) showed an altered dorsoventral patterning of the somites in response to retrovirally injected

*Wnt-1* in the paraxial mesoderm. Their findings are similar to ours with regard to up-regulation of *Pax-3* and loss of *Pax-1* expression. Contradictory is the fact that they did not observe any alterations in the expression pattern of the myogenic markers, while we found strong amplification of *MyoD* expression. We suggest that this discrepancy could be due to differences in the level of Wnt-1 produced by retroviral infection and cell transplantation. Additionally, the position of the Wnt signal relative to the somite seems to play a critical role. Thus, we also did not find enhanced myogenesis if we implanted the Wnt-secreting cells under the ectoderm (data not shown).

### *Wnts Influence Somite Epithelialization*

*Paraxis* expression is a prerequisite for somite epithelialization (Burgess *et al.*, 1996; Barnes *et al.*, 1997; Sosic *et al.*, 1997). Ectopically implanted Wnt-secreting cells (Wnt-1, -3a, -4) up-regulate the expression of *Paraxis* and promote somite epithelialization. Thus *Paraxis* may be a target gene of the *Wnts*. This idea was supported by findings of Sosic *et al.* (1997) who found *Paraxis* expression to depend on the neural tube and the surface ectoderm. They were able experimentally to switch off *Paraxis* expression and to block dermomyotome formation. Mice lacking *Paraxis* do not form somite epithelium and show severe defects of muscle (Burgess *et al.*, 1995, 1996).

One way in which the Wnts could influence somite epithelialization is through their suggested involvement in cell adhesion. The *Wnt* signal transduction pathway leads to an accumulation of β-catenin (Hinck *et al.*, 1994). In its role as a cell adhesion molecule, β-catenin links the cadherins to the cytoskeleton (Wheelock and Knudsen, 1991), and the cadherins represent key molecules in the process of somite epithelialization (Duband *et al.*, 1987). Additionally, the expression of *E-cadherin* depends on the activation by the TCF/LEF complex (Shimamura *et al.*, 1994; Huber *et al.*, 1996b). Linask *et al.* (1998) described the expression of *N-cadherin* and *β-catenin* in the myotome. Therefore, cadherins may be involved in the formation of the epithelial dermomyotome.

### *Wnts Diminish the Sclerotome-Inducing Effect of Shh*

In our experiments, *Wnt*-expressing cells in close contact with the notochord reduced the amount of sclerotome formed, presumably by interfering with the inductive effect of Shh. Implantation of a supernumerary notochord, or the retroviral overexpression of Shh, results in altered somite patterning. The expression of *Pax-3* is lost and the whole somite expresses *Pax-1* (Pourquié *et al.*, 1993; Brand-Saberi *et al.*, 1993; Johnson *et al.*, 1994).

Conversely, several groups have found that Shh is required for myogenesis (Münsterberg *et al.*, 1995; Stern *et al.*, 1995; Buffinger and Stockdale, 1995; Cossu *et al.*, 1996; Pownall *et al.*, 1996; Dietrich *et al.*, 1997; Maroto *et al.*, 1997; Borycki *et al.*, 1998; Duprez *et al.*, 1998; Tajbakhsh *et al.*, 1998; Cann *et al.*, 1999). Therefore, a dual function of Shh has been suggested. A low concentration of Shh far from the protein source might cause a different response of somitic cells from high concentrations in close proximity to the notochord. Borycki *et al.* (1998) have suggested that different groups of somitic cells might transduce the Shh signal in different ways. They found differences in expression of *Gli* transcriptional factors and *Patched* receptors to be responsible for the dual effect of Shh. *Gli* and *Patched* are expressed in the sclerotome under the influence of the notochord and transduce the ventralizing signal to the sclerotome. *Gli 2/4* are expressed in the dorsal somite under the influence of the ectoderm and transduce the Shh signal to the myogenic lineage. Another function of Shh was recently presented by Lee *et al.* (2000). They showed that Shh induces in the sclerotome the expression of Sfrp2, a known Wnt antagonist, which could bind Wnt-1 and Wnt-4, thus opposing Wnt-induced myogenesis. Our findings extend this observation. The elevated Wnt dosage in the sclerotome from the ectopic source may overwhelm the secreted Wnt antagonist and initiate Wnt binding to their receptors on ventral somitic cells. Thus, Wnts are able to antagonize the notochord-derived Shh signal in a dosage-dependent manner. Additional evidence for the dosage-dependent antagonism between Shh and Wnts derives from the study of Fan *et al.* (1997). They cultured unsegmented paraxial mesoderm explants between aggregates of Shh- and Wnt-secreting cells and found the mesodermal reaction to these "neighbors" to depend on the ratio of Shh to Wnts.

### *Wnts Are Part of a Gene Activation Cascade in Myogenesis*

Reshef and colleagues (1998) found an elevation in *Pax-3* expression, a marker for myogenic precursors in the dermomyotome, in somites cocultured with dorsal neural tube or surface ectoderm. Expression of *Wnt-1* and *-3a* in the dorsal neural tube are induced by BMP4 via the overlying ectoderm (Dickinson *et al.*, 1995; Marcelle *et al.*, 1997). In experiments described here, the implantation of Wnt-1-, -3a-, and -4-secreting cells led to an up-regulation of *Pax-3* expression. These results suggest that Wnts act as upstream regulators of *Pax-3*, possibly via the activity of *Paraxis*. That *Pax-3* is at least in part regulated by *Paraxis* was recently shown by Wilson-Rawls *et al.* (1999). In *Paraxis*-null mutants, *Pax-3* expression is reduced in the first somites and lost in compartmentalized somites, especially in precursors of the nonmigratory hypaxial muscle (Burgess *et al.*, 1996; Wilson-Rawls *et al.*, 1999). The incomplete loss of *Pax-3* in the *Paraxis*-null mutant could be explained by the finding of Barber *et al.* (1999) that there exist different isoforms of *Pax-3* in the migratory and in the nonmigratory myogenic lineages which might be induced via different mechanisms.

*Pax-3* plays a fundamental role in the determination of myogenic cells (Franz *et al.*, 1993; Bober *et al.*, 1994b;

Goulding *et al.*, 1994; Williams and Ordahl, 1994; Tremblay *et al.*, 1998; Henderson *et al.*, 1999). Strong evidence for the role of *Pax-3* as an upstream regulator of *MyoD* expression was presented by Tajbakhsh *et al.* (1997), Maroto *et al.* (1997), and Henderson *et al.* (1999). We found an enlarged *MyoD*-positive myotome after implantation of *Wnt-1-, -3a-, and -4*-expressing cells. Therefore, the role of Wnts in regulating myogenesis within the somite may be as an upstream regulator of *MyoD* expression, indirectly via the expression of *Pax-3*. Since neither a Pax-3- nor a TCF-binding site has been found in the *MyoD* locus (Kucharczuk *et al.*, 1999), it is likely that intermediate factors may be necessary to activate *MyoD* expression.

### *The Size of the Myotome May Be Controlled by Several Mechanisms*

The enlargement of the myotome observed after ectopic implantation of Wnt-secreting cells could be due to one or more factors. First, an increase in cell proliferation or a decrease in apoptosis of myogenic precursors could lead to and expanded myotome. However, in experiments not shown, we found no indication for such a mechanism. Another source of the additional myotomal cells could be the dermomyotome itself. In addition to myogenic precursors, the dermomyotome contains cells which give rise to dermis and blood vessels. Conceivably, these nonmyogenic cells could be transformed into myogenic precursor cells by a Wnt-controlled mechanism.

Finally, the ventral compartment of the somite could be another source of the additional muscle cells. In several cell culture studies the muscle-inducing ability of diverse embryonic structures was examined separately. In accordance with our results, somites exhibited myogenesis only if cultured in contact with *Wnt*-expressing structures such as neural tube and surface ectoderm (Vivarelli and Cossu, 1986; Kenny-Mobbs and Thorogood, 1987; Buffinger and Stockdale, 1994; Stern and Hauschka, 1995; Angello *et al.*, 1997). This promoting effect on dermomyotome formation and myogenesis was mimicked by *Wnt*-expressing cells in culture experiments (Münsterberg *et al.*, 1995; Stern *et al.*, 1995; Fan *et al.*, 1997). These authors observed an increase in myogenesis if Wnts acted in concert with Shh. Our results show that the enlargement of the dorsal compartment takes place at the expense of the ventral compartment. This suggests that the ventralizing effect of Shh could be diminished by the influence of dorsalizing Wnts *in vivo*.

Spence *et al.* (1996) and Dietrich *et al.* (1997) also proposed that the dorsal neural tube and notochord have opposing functions on somite myogenesis. Spence *et al.* (1996) showed that rotation of the neural tube leads to the formation of a secondary dermomyotome in the ventral somite. At the time of cell grafting, in experiments presented here, cells in the ventral part of the somite are not committed to the sclerotomal lineage (Aoyama and Asamoto, 1988; Dockter and Ordahl, 1998). Our results are consistent with a model in which cells of the ventral somite are able to switch to a myogenic fate in response to Wnt signals emanating from the dorsal neural tube.

## CONCLUSION

1. Somitic epithelium is likely induced and maintained by Wnts and might promote myogenesis.
2. Augmentation of the Wnt signal leads to an enlarged myotome.
3. Ectopically secreted Wnts diminish the sclerotome-inducing effect of Shh.
4. Wnts represent important signaling molecules for myogenic induction.
5. Wnts promote expression of *Paraxis*, *Pax-3*, and *MyoD*.

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